



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : A61K 45/06	A2	(11) International Publication Number: WO 00/40269 (43) International Publication Date: 13 July 2000 (13.07.00)
(21) International Application Number: PCT/US00/00191 (22) International Filing Date: 5 January 2000 (05.01.00) (30) Priority Data: 60/114,906 5 January 1999 (05.01.99) US (71)(72) Applicants and Inventors: LEE, Clarence, C. [US/US]; 1141 Kelvington Way, Lilburn, GA 30047 (US). LEE, Feng-Min [US/US]; 1141 Kelvington Way, Lilburn, GA 30047 (US). (74) Agents: PABST, Patrea, L. et al.; Arnall Golden & Gregory, LLP, 2800 One Atlantic Center, 1201 West Peachtree Street, Atlanta, GA 30309-3450 (US).		(81) Designated States: AU, CA, CN, JP, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>Without international search report and to be republished upon receipt of that report.</i>
(54) Title: PHARMACEUTICAL COMPOSITIONS FOR TREATMENT OF DISEASED TISSUES (57) Abstract <p>A method to treat diseased tissue is provided where a cytotoxic compound is administered to a patient in need of treatment in combination with an immunostimulant. Diseased cells and/or infectious microbes/viruses are killed by the cytotoxic compound in the presence of the immunostimulant. The cell components including cellular contents and cell membrane fragments are presented by the immunostimulant to the host animal as antigens to stimulate the immune responses toward other diseased cells of the same type(s), that either remain in the vicinity or reside in distant tissues or organs. The cytotoxic molecule and immunostimulant are preferably applied locally at high concentrations, either sequentially or, preferably, simultaneously. For example, the composition can be administered directly to a target cancer. The composition can be prepared in various forms, such as a paste, a time release molded solid shape, a solution, a mixture with emulsifier, etc. Alternatively, the cytotoxic molecule and immunostimulant are applied in sequence.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

PHARMACEUTICAL COMPOSITIONS FOR TREATMENT OF DISEASED TISSUES

Field of the Invention

The present invention generally relates to pharmaceutical compositions for the treatment of diseased tissues and organs.

Background of the Invention

Diseased tissues in animals such as bacterially infected abscesses, virally inflamed tissues, and various tumors are usually associated with bacterial and/or viral infection or viruses, and/or unregulated cellular activities such as inflammation.. Abscesses are usually due to bacterial infection. The infection initially causes localized host tissue inflammation and immune responses. Abscesses are extremely difficult to treat systemically. Moreover, should the treatment fail, fatal breakthrough infection can result. Virally inflamed tissues and organs are also difficult to treat.

Solid, soft-tissue malignant tumors are perhaps the most problematic diseases in animals. A malignant tumor (cancerous tumor) is abnormal animal tissue consisting of cells that grow by cellular proliferation more rapidly than normal. In addition, the cells can metabolize, when the cells invade the surrounding inter-cellular matrix, such as collagen basement membrane, and escape to distant sites where secondary tumors grow. Malignant tumors can develop in any tissue of any organ in animals of any age. When an unequivocal diagnosis of a malignant tumor is made, treatment decisions become preeminent. Though no single treatment approach is applicable to all malignant tumors, successful therapy must be focused on both the primary and secondary tumors, whether clinically apparent or microscopic. Most of the malignant tumors in animals are usually surgically removed, the area irradiated with radiation, and/or treated with systemic infusion of cytotoxic compounds. All of these methods have some critical shortcomings and/or limitations under certain circumstances.

Surgery frequently cannot remove all cancerous cells/tissues in the host animal because of either their physical location or difficulty in detection of

multiplicity of cancers. For example, it is extremely difficult or impossible to remove glioblastoma completely, due to the need to preserve surrounding non-diseased brain cells so that the patient will have a good quality of life post-surgically. For another example, multiple bone-cancer sites cannot be efficiently treated with radiation and/or surgery because the patient cannot physically tolerate major radiation therapies and/or surgeries within a short period of time. External-beam radiation therapy and surgery are expensive and time-consuming in addition to their significant risks and permanent adverse effects.

Radiation therapy plays a key role in the remediation of early stage breast cancer, germ-cell tumors, squamous cell carcinoma of the head and neck, Hodgkin's disease, nodular and diffuse non-Hodgkin's lymphomas, early stage non-small cell lung cancer, and seminoma. Non-invasive, external beam and interstitial irradiation of diseased cancers are also used as an adjuvant or palliative treatment of diseased tissues. Irradiation cannot always destroy all cancerous cells within a tumor locus. In addition, irradiation can terminally damage surrounding non-diseased tissues, such as the vocal cords and the epithelium of gastrointestinal tract.

Systemic infusion of cytotoxic compounds, which function by preventing cell division (mitosis), development, maturation, or spread of cancer cells, are used frequently as first-line therapy for various cancers. They include classes of alkylating agents, antibiotics, antimetabolites, inorganic ions, enzymes, enzyme inhibitors and hormone analogs that competitively bind on the receptor sites of the cancer cells. Alkylating agents include nitrosourea and analogs, nitrogen mustard and analogs, plant-derived alkaloids and some chemicals known to bind nonspecifically to DNA, RNA and protein to stop cell growth. Many classic antibiotics for the treatment of microbial infections, such as doxorubicin, bleomycin, dactinomycin, idarubicin, and mitomycin, that bind DNA, and inhibit DNA, RNA and protein synthesis are also cytotoxic toward mammalian cells. Since antimetabolites include compounds that inhibit folic acid metabolism and the synthesis of DNA, RNA and protein, some could be

classified as alkylating agents or antibiotics.

Cancers of the testis and small-cell carcinoma of the lung and early stages of choriocarcinoma, Hodgkin's disease, leukemia, Burkitt's lymphoma, and diffuse large cell lymphoma have been found to be "curable" using systematic chemotherapy. However, the systemic toxicity and adverse effects of these compounds generally limit their utilization at high levels for other cancers. Hair loss, nausea, cytotoxicity of normal cells, cardiotoxicity and loss of appetite are a few common examples of the adverse effects of chemotherapy.

Despite use of the above "drug" therapies, many initially localized cancers, such as brain, lung, colon, esophagi, and melanoma, remain difficult to treat, and are often fatal. A patient's quality of life deteriorates significantly while undergoing one or more surgical procedures, chemotherapeutic treatments, and/or regimens of surgical procedures, chemotherapeutic treatments and/or radiation therapy. The treatment outcome of each case is also unpredictable.

Various attempts have been made to treat cancer more effectively. Other potential treatments include hormonal therapies for cancers that require the binding of estrogen or testosterone, and immunotherapies. Hormonal therapies that are palliative and adjuvant in nature cannot cure cancers. Systemic combination immunotherapy using *Bacillus calmette-guerin* (BCG) with other therapies has not been demonstrated to be effective for treating cancer, for example, as reported non-small cell carcinoma of the lung (Maurer, LH et al. J Clin Oncol. 3(7):969 (1985)).

U.S. Patent No. 4,340,586 to Bekierkunst et al. discloses the use of cell walls of mycobacteria (such as BCG) in combination with cord factor to stimulate the host immune system and treat skin disorders. U.S. Patent Nos. 4,744,984 and 4,503, 048 also disclose the use of mycobacterial cell wall for the treatment of viral infections and tumors.

Mycobacterium bovis (such as strain BCG), a non-specific immunostimulant, traditionally has been used to vaccinate tuberculin negative patients against tuberculosis. The CpG motif of BCG has a primary effect on

the T cell. It also increases natural killer (NK) cell tumoricidal activity, induces the synthesis of macrophage activating factors and interferons, and inhibits tumor growth. Tokunaga et al, Microbiol. Immunol. **36**(1):655-666 (1992); and Klinman et al., Proc. Natl. Acad. Sci. USA 93:2879-2883 (1996). Nonviable preparations of *Corynebacterium parvum*, another nonspecific immunostimulant, have remained an investigational agent due to lack of evidence to demonstrate benefit.

The above immunotherapies have not been proven effective toward various bacterial and viral infections and tumors. Accordingly, it is an object of the invention to provide improved compositions and methods for treatment of diseased tissue resulting for bacterial or viral infection and cancer.

Summary of the Invention

A method to treat diseased tissue is provided where a cytotoxic compound is administered to a patient in need of treatment in combination with an immunostimulant. Diseased cells and/or infectious microbes/viruses are killed by the cytotoxic compound in the presence of the immunostimulant. The cell components including cellular contents and cell membrane fragments are presented by the immunostimulant to the host animal as antigens to stimulate the immune responses toward other diseased cells of the same type(s), that either remain in the vicinity or reside in distant tissues or organs.

The cytotoxic molecule and immunostimulant are preferably applied locally at high concentrations, either sequentially or, preferably, simultaneously. For example, the composition can be administered directly to a target cancer. The composition can be prepared in various forms, such as a paste, a time release molded solid shape, a solution, a mixture with emulsifier, etc. Alternatively, the cytotoxic molecule and immunostimulant are applied in sequence. Generally, the concentration of each agent is preferably at a minimum of 150% of its concentration in serum when administered via appropriate oral, i.v. or i.m. route. In the event that systemic administration of the cytotoxic agent at an effective dose is neuro-toxic, organ-toxic or fatal, its local application at a

minimum of 1 microgram per deciliter with an immunostimulant offers efficacy with lessened or no adverse effects. Conversely, in the event that systemic administration of the immunostimulant, such as viable BCG, is harmful or fatal, its local application at a minimum of 1 microgram per deciliter with a cytotoxic compound also offers efficacy with little or no adverse effects.

Detailed Description of the Invention

A pharmaceutical composition comprising a cytotoxic compound and an immunostimulant is described. The cytotoxic compound attacks the diseased animal cell and/or infectious microbe, such as a bacterium, fungus or protozoan, causing it to lyse. The immunostimulant raises the level of activity of the immune system, causing greater production of immune components against the lysed cell and its contents. The composition is preferably applied locally for treatment of diseased tissues and organs. Because a small volume of highly concentrated composition is directly applied to the target tissues and/or organs, it exerts high cytotoxicity and immune stimulation in a small area. Any leakage throughout the host body, is at a level where the systemic concentration of the composition is several magnitudes lower than those of the two active ingredients administered via an intravenous or oral route. The method described herein involves application of the cytotoxic compound and immunostimulant to diseased tissue, preferably as one formulation or by simultaneous administered. However, the compound may be applied sequentially.

Compositions

Cytotoxic Compounds

Cytotoxic molecules include compounds that kill or inhibit replication of virus, bacteria, protozoa, fungi, and/or eucaryotic cells. Examples include alkylating agents. Other compounds include antimetabolites, inorganic ions, metal chelators, enzymes, enzyme inhibitors, hormone analogs, organic compounds, platinum complexes, and antineoplastic compounds. Exemplary compounds are described below.

Alkylating agents and analogs include nitrosourea and analogs, nitrogen

mustard and analogs, methanesulfonate and analogs, epoxide and analogs, plant-derived alkaloids and some chemicals known to bind non-specifically to DNA, RNA, and protein to stop cell growth. These chemicals that alkylate DNA and RNA as well as inhibit key enzymatic reactions by carbamoylation of amino acids in proteins are generally cytotoxic toward both mammalian and microbial cells. Examples of such compounds include camustine, lomustine, cyclophosphamide, etoposide, gemcitabine hydrochloride, , altretamine, ifosfamide, chlorambucil, procarbazine hydrochloride, busulfan, cyclophosphamide, vincristine sulfate, carboplatin, cisplatin, vinblastine sulfate, and streptozocin, dichloroethyl ifosfamide, dianhydromannitol diepoxide, and ethylenimine.

Many classic antibiotics for the treatment of microbial infections that bind DNA, and inhibit DNA, RNA and protein synthesis are also cytotoxic toward mammalian cells. Examples of such antibiotics include aminoglycosides amphenicols, ansamycins, beta-Lactams, lincosamides, macrolides, polypeptides, and tetracyclines. Examples include cycloserine, mupirocin, tuberin, doxorubicin hydrochloride, bleomycin sulfate, daunorubicin hydrochloride, dactinomycin, doxorubicin hydrochloride, idarubicin hydrochloride, mitomycin, porfiromycin, and their derivatives.

Examples of antifungals include Amphotericin, flucytosine, fluconazole, Grifulvin, Gris-PEG, griseofulvin, terbinafine HCl, ketoconazole and Itraconazole.

Anti-ameobic compounds include acedapsone, amodiaquin, arsthinol, arteether, artemether, artemisinin, artesunate, atovaquone, bebeerine, berberine, bialamicol, carbarsone, cephaeline, chirata, chlorbetamide, chlorguanide, chloroquine, chlorphenoxamide, chlorproguanil, chlortetracycline, cinchona, cinchonidine, cinchonine, cycloguanil, dehydroemetine, dibromopropamidine, diloxanide, diphetarsone, disodium arsenate, emetine, fumagillin, gentiopicrin, glaucarubin, glycobiarsol, halofantrine, hydroxychloroquine, 8-hydroxy-7-iodo-5-quinoline-sulfonic acid, iodochlorohydroxyquin, iodoquinol, mefloquine

hydrochloride, 3- methylarsacetin, pamaquine, paremomyacin, phanquinone, plasmocid, polybenzarsol, primaquine, propamidine, pyrimethamine, quinacrine, quinfamide, quinidine, quinine, quinocide, quinoline, secnidazole, sulfarside, teclozan, thiocarbamizine, thiocarbarsone, and tinidazole. Some of these compounds are also classified as antimetabolites, antibiotics, or in other categories.

Antiseptics include alcohols, aldehydes, dyes, guanidines, halogens/Halogen containing compounds, mercurial compounds, nitrofurans, peroxides/permanganates, phenols, quaternary ammonium Compounds, quinolines, silver compounds, and aluminum acetate and subacetate.

Antiviral agents include purines/pyrimidinones and others such as acemannan, acetylleucine monoethanolamine, amantadine, amidinomycin, delavirdine, foscarnet sodium, indinavir, interferons, kethoxal, lysozyme, methisazone, moroxydine, nevirapine, podophyllotoxin, ribavirin, rimantadine, ritonavir, saquinavir, stallimycin, statolon, tromantadine, xenazoic acid, etc.

Since antimetabolites include compounds that inhibit folic acid metabolism and the synthesis of DNA, RNA and protein, some could be classified as alkylating agents or antibiotics. Others are purine analogs and pyrimidine analogs. The most common ones are cytarabine (Cytosar-U[®]), floxuridine (FUDR[®]), fludarabine phosphate (Fludara U[®]), fluorouracil (Efudex U[®]), hydroxyurea (Hydrea[®]), mercaptopurine (Purinethol[®]), methotrexate sodium, pentostatin (Nipent[®]), picamycin (Mithracin[®]), and thioguanine (Tabloid[®]), and mitotane (Leustatin[®]), Triphosphate (2-fluoro-ara-ATP), pentostatin, cladribine, and their derivatives

Inorganic compounds, such as caustic acids and bases, zinc nitrate, and cesium chloride at high concentration are also cytotoxic to both mammalian and/or microbial cells.

Metal chelators include deferoxamine, sodium ditiocarb, calcium disodium edetate, disodium edetate, sodium edetate, trisodium edetate, penicillamine, calcium trisodium pentetate, pentetic acid, succimer and trientine.

Enzymes, such as asparaginase, that reduce the availability of certain exogenous amino acids or nutrients that are required for survival of malignant cells are also cytotoxic. Enzyme inhibitors that irreversibly bind to the active sites of important enzymes that are required in certain important metabolic pathway of malignant cells are cytotoxic.

Platinum complexes include carboplatin, cisplatin, miboplatin, and oxaliplatin.

Hormone analogs that competitively bind on the receptor sites of the cancer cells could also be useful as cytotoxic compounds. Useful hormones and hormone analogs include anastrozole, flutamide, nilutamide tamoxifen sulfate, diethylstilbestrol, and chlorotrianisene.

Antineoplastic drugs and analogs that do not belong to the above categories include aceglatone, amsacrine, bisantrene, defosfamide, demecolcine, diaziquone, eflornithine, elliptinium acetate, etoglucid, fenretinide, hydroxyurea, lonidamine, miltefosine, mopidamol, nitracrine, pentostatin, phenamet, podophyllinic acid 2-ethyl-hydrazide, procarbazine, razoxane, sobuzoxane, spirogermanium, tenuazonic acid, triaziquone, 2, 2, 2-trichlorotriethylamine, and urethan. Examples of commonly used antineoplastics include Camptosar, betamethasone sodium and DTIC-Dome.

Other potential new cytotoxic agents in development include adozelesin, altretamine, aminopterin, anthracycline, AR102, Arimidex, 5-azacytidine, 5-aza-2-deoxycytidine, bisnafide, 2B1 bispecific murine Mab, bizelestin, Bropirimine, bryostatin 1, BUDR, Campath-1H, capecitabine, Catrix, chloroquinoxaline sulfonamide, CI-980, cordycepin, corticotropin releasing factor, crisnatol, daunorubicin citrate liposome injection, DHAC, diethyl homospermine, distamycin, DFMO, DPPE, droloxifene, EGF fusion toxin, anti-EGFr chimeric Mab, EGM fusion toxin, amifostine, exemestane, filgrastim, Filmix, gallium nitrate, gemcitabine, glucosamylmuramyl tripeptide dipalmitoylglycerol (Theramide), guanine arabinoside, homoharringtonine (HHT), idarubicin, idoxifene, irinotecan, IUDR, LDI-200, letrozole, liarozole, Linomide

roquinimex, MDX-447, MDX-H210, mitalactol, mitoguazone (Zyrkamine), mitoxantrone, Neu-Sensamide, octreotide pamoate, OncoSPECT (BR-Tc), ONYX-015, oxaliplatin, Panretin, paclitaxel, peldesine, phenylacetate, N-phosphonoacetyl-L-aspartic acid, piritrexim, porfiromycin, pyrazine, Radinyl etanidazole, 9-cis-retinoic acid, RMP-7, SU101, Temodal, thalidomide, Tirazone, TBC-CEA, thalidomide, Tirazone, toremifene, VX-710, trimetrexate glucuronate, topotecan, radiolabeled Mab CC-49, thiotepa for injection, etc.

Preferred cytotoxic compounds are alkylating agents such as improsulfan, carboquone, triethylenemelamine, chlorambucil, uracil mustard, carmustine, and mitobronitol, classic antibiotics, such as bleomycin, fluorouracil, mitomycin, and duxorubicin, antifungal agents, such as amphotericin, flucytosine, fluconazole, griseofulvin, terbinafine HCl, ketoconazole and itraconazole, antiamebic compounds, antiseptics, such as ethyl alcohol, formaldehyde, glutaraldehyde, acriflavine, aminacrine, brilliant green, ethacridine, gentian violet, magenta I, methyl blue, alexidine chlorohexidine, picloxydine, calcium iodate, iodic acid, iodine, povidone-iodine, mercurous acetate, thimersal, nitrofurazone, hydrogen peroxide, magnesium peroxide, zinc permanganate, chloroxylonol, cresol, benzethonium chloride, cetylpyridinium chloride, chloroxine, 8-hydroxyquinoline, iodochlorhydroxyquin, silver lactate, silver nitrate, m-cresyl acetate, and boric acid, antiviral agents, such as amidinomycin, delavirdine, dideoxyadenosine, floxuridine, indinavir, kethoxal, methisazone, moroxydine, podophyllotoxin, ribavirin, stallimycin and xenazoic acid, antimetabolites, inorganic compounds, such as hydrochloric acid, sulfuric acid, nitric acid, sodium hydroxide, silver nitrate, and cesium chloride, organic compounds, such as acetic acid, formic acid, L-ascorbic acid, ethanol, isopropanol, and DMSO, metal chelators, enzymes, enzyme inhibitors, hormone analogs, platinum complexes, such as carboplatin, cisplatin, miboplatin, and oxaliplatin, and other antineoplastic compounds, such as paclitaxel, vinblastine, amsacrine, hydroxyurea, tenuazonic acid and urethan.

Particularly preferred cytotoxic compounds include acetic acid, ethanol,

BiCNU and classic antibiotics such as bleomycin, fluorouracil, mitomycin, and duxorubicin.

Immunostimulants

Immunostimulants include molecules and complexes of molecules, such as pyrogens, exotoxins, bacterial cell wall fragments or the initial microorganisms, plant and animal allergens, mammalian cytokines, animal venom, and mucopolysaccharides which elicit or enhance an immune response characterized by activation of immune cells (i.e. T or B cells, macrophages, or monocytes), as production of antibody as immunomodulators such as cytokines. Mucopolysaccharides include chondroitin sulfates A, B, and C, hyaluronic acid, and other glycosaminoglycans. Pyrogens include lipid A molecule, and fragments of pyrogen from various Gram negative bacteria. Exotoxins include all toxins excreted by microorganisms. Bacterial cell walls include whole, fragments and components of the cell wall of microorganisms such as *Mycobacterium bovis*. For example, synthetic oligonucleotides derived from *Bacillus calmette-guerin* (BCG) as taught in Tokunaga et al, Microbiol. Immunol. **36**(1):655-666 (1992) can be used. Pieces of cell walls of microorganisms can be generated, for example, by mechanical shearing, thermal denaturation or chemical disruption of microbial cell walls. Other bacteria such as *Corynebacterium parvum*, *C. diphtheria*, *smegmatis*, *M. phlei*, *M. cansasii*, *M. tuberculosis*, *Nocardia rubra*, and *asteroides*, and cell wall extracts therefrom, as described in U.S. Patent No. 4,503,048, can be used. Other bacteria that can be used include *Bordatella pertussis*, *Eubacterials*, *avium*, *fortuitum*, *kansasii*, *smegmatis*, *vaccae*, *rubra*, and *Rhodococcus*. Muramyl dipeptide (N-acetylmuramyl-L-alanyl-D-isoglutamine) and derivatives of mycolic acids are also examples of bacterial cell wall components.

Some Deoxyribonucleic acids and ribonucleic acids of animals and plants can be used for example, the CpG motifs taught in Klinman et al. Proc. Natl. Acad. Sci. USA 93:2879-2883 (1996).

Microorganism which can be used include intact, live, damaged and dead

bacteria, including BCG, yeast/fungi, and viruses. Representation Plant allergens include carbolic acid, *Acer negundo*, *Agrostis alba*, *Alnus incana*, *Ambrosia elatior*, *Artemisia tridentata*, *Betula alba*, *Bromus inermis*, *Cynodon dactylon*, *Dactylis glomerata*, *Fraxinus pennsylvanica*, *Iva xantifolia*, *Juglans nigra*, *Juniperus scopulorum*, *Kochia scoparia*, *Poa pratensis*, *Populus nigra italica*, *Quercus rubra*, *Secale cereale*, *Sorghum halepense*, *Ulmus pumilazea mays*, poison hemlock, poison ivy, poison oak. Representation Mammalian cytokines include, interferons, interleukins, and granulocyte-macrophage colony stimulating factor. Animal venoms can be derived from ants, bees, centipedes, cone shell, Gila monster, jellyfishes (5-hydroxytryptamine), keyhole limpet (hemocyanin), kissing bug, millipedes, mosquitoes, puss caterpillar, scorpions, scorpion fish, sea anemone, sea urchins, snakes, spiders, spotted octopus, starfishes, stonefish, and weever fish.

Preferred immunostimulants include Isoprinesine, endotoxins, cytokines, Freund's adjuvants, BCG, and bacterial cell wall fragments such as from BCG, active and inactivated bacteria, such as *Corynebacterium parum*, macrophage stimulating factors such as granulocyte-macrophage colony stimulating factor, lipid A containing molecules, such as pyrogen from gram-negative bacteria, cytokines such as interleukins, natural or synthetic DNA/RNA fragments, such as oligonucleotides containing CpG motif, animal venom such as fire ant venom, plant allergens such as carbolic acid, synthetic immunostimulants such as Levamisole, and mucopolysaccharides such as chondroitin sulfate A.

A particularly preferred immunostimulant include bacterial cell wall fragments such as from BCG.

Pharmaceutical Carriers

Suitable pharmaceutical carriers are known to those skilled in the art. For example, when the active ingredient is administered parenterally, in sterile liquid dosage forms, the carrier can be water, suitable oil, saline or other buffered physiological solution, aqueous dextrose or related sugar solutions and glycols, such as propylene glycol or polyethylene glycol. Solutions for

parenteral administration preferably contain a water soluble form of the active ingredient, suitable stabilizing agents, and if necessary, buffer substances. Antioxidizing agents such as sodium bisulfite, sodium sulfite, or ascorbic acid either alone or combined are suitable stabilizing agents. Also used are citric acid and its salts and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as benzalkonium chloride, methyl- or propylparaben, and chlorobutanol. Suitable pharmaceutical carriers can be included and are described in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Co., Easton, PA, p.1418 (1985).

Methods for making the compositions

Immunostimulants can be prepared as described in the prior art, such as U.S. Patent Nos. 4,744,984 to Ragland and 4,503,048 to Cantrell. Cytotoxic compounds can be prepared as known in the art. The compounds can be simply mixed together, preferably in a pharmaceutically acceptable carrier.

Buffers, acids, and bases can be used to adjust the pH of the composition. Agents to increase the diffusion distance of agents released from the implanted polymer can also be included. Surfactants may be necessary in implant formulations to enhance wettability of poorly soluble or hydrophobic materials. Surfactants such as polysorbates or sodium lauryl sulfate are, if necessary, used in low concentrations, generally less than 5%. Binders are adhesive materials that are incorporated in implant formulations to bind powders and maintain implant integrity. Binders may be added as dry powder or as solution. Sugars and natural and synthetic polymers may act as binders. Materials added specifically as binders are generally included in the range of about 0.5 to 15% w/w of the implant formulation. Certain materials, such as microcrystalline cellulose, also used as a spheronization enhancer, also have additional binding properties.

Various coatings can be applied to modify the properties of the implants. Three types of coatings are seal, gloss and enteric. The seal coat prevents excess moisture uptake by the implants during the application of aqueous based enteric

coatings. The gloss coat improves the handling of the finished product. Water-soluble materials such as hydroxypropyl cellulose can be used to seal coat and gloss coat implants. The seal coat and gloss coat are generally sprayed onto the implants until an increase in weight between about 0.5% and about 5%, preferably about 1% for seal coat and about 3% for a gloss coat, has been obtained.

In the preferred embodiment, only the cytotoxic compound and immunostimulant to be released are incorporated into the delivery device, although other biocompatible, preferably biodegradable or metabolizable, materials can be included for processing purposes as well as additional therapeutic agents. More than one cytotoxic compound and immunostimulant can be used.

Controlled release devices are typically prepared in one of several ways. For example, the polymer can be melted, mixed with the substance to be delivered, and then solidified by cooling. Such melt fabrication processes require polymers having a melting point that is below the temperature at which the substance to be delivered and polymer degrade or become reactive. Alternatively, the device can be prepared by solvent casting, where the polymer is dissolved in a solvent, and the substance to be delivered dissolved or dispersed in the polymer solution. The solvent is then evaporated, leaving the substance in the polymeric matrix. Solvent casting requires that the polymer be soluble in organic solvents and that the agents to be encapsulated be soluble or dispersible in the solvent. Similar devices can be made by phase separation or emulsification or even spray drying techniques. In still other methods, a powder of the polymer is mixed with the active compounds and then compressed to form an implant. Other delivery systems including films, coatings, pellets, slabs, and devices can be fabricated using solvent or melt casting, and extrusion, as well as standard methods for making composites.

Other delivery systems including films, coatings, pellets, slabs, and devices can be fabricated using solvent or melt casting, and extrusion, as well as

standard methods for making composites.

Microspheres can be prepared using any of the methods developed for making microspheres for drug delivery, for example, as described by Mathiowitz and Langer, J. Controlled Release 5,13-22 (1987); Mathiowitz, et al., Reactive Polymers 6, 275-283 (1987); and Mathiowitz, et al., J. Appl. Polymer Sci. 35, 755-774 (1988). The selection of the method depends on the polymer selection, the size, external morphology, and crystallinity that is desired, as described, for example, by Mathiowitz, et al., Scanning Microscopy 4,329-340 (1990); Mathiowitz, et al., J. Appl. Polymer Sci. 45, 125-134 (1992); and Benita, et al., J. Pharm. Sci. 73, 1721-1724 (1984). Methods routinely used by those skilled in the art include solvent evaporation, hot melt encapsulation, solvent removal, spray drying, phase separation and ionic crosslinking of gel-type polymers such as alginate or polyphosphazines or other dicarboxylic polymers to form hydrogels.

The microparticles can be suspended in any appropriate pharmaceutical carrier, such as saline, for administration to a patient. In the most preferred embodiment, the microparticles will be stored in dry or lyophilized form until immediately before administration. They will then be suspended in sufficient solution for administration. The polymeric microparticles can be administered by injection, infusion, implantation, orally, or administration to a mucosal surface, for example, the nasal-pharyngeal region and/or lungs using an aerosol. The other devices are preferably administered by implantation in the area where release is desired. Lower dosages are used with implantable controlled release devices than with other forms of administration.

Biocompatible polymers can be categorized as biodegradable and non-biodegradable. Biodegradable polymers degrade *in vivo* as a function of chemical composition, method of manufacture, and implant structure. Synthetic and natural polymers can be used although synthetic polymers may be preferred due to more uniform and reproducible degradation and other physical properties. Examples of synthetic polymers include polyanhydrides, polyhydroxyacids such

as polylactic acid, polyglycolic acid and copolymers thereof, polyesters, polyamides, polyorthoesters, and some polyphosphazenes. Examples of naturally occurring polymers include proteins and polysaccharides such as collagen, hyaluronic acid, albumin, and gelatin. The ideal polymer must be processible and flexible enough so that it does not crumble or fragment during use.

Use of polyanhydrides in controlled delivery devices is described in U.S. Patent No. 4,857,311 to Domb and Langer, U.S. Patent No. 4,888,176 to Langer, et al., and U.S. Patent No. 4,789,724 to Domb and Langer. Other polymers such as polylactic acid, polyglycolic acid, and copolymers thereof have been commercially available as suture materials for a number of years and can be readily formed into devices for drug delivery.

Non-biodegradable polymers remain intact *in vivo* for extended periods of time (years). Agents loaded into the non-biodegradable polymer matrix are released by diffusion through the polymer's micropore lattice in a sustained and predictable fashion, which can be tailored to provide a rapid or a slower release rate by altering the percent loading, porosity of the matrix, and implant structure. Ethylene-vinyl acetate copolymer (EVAc) is an example of a nonbiodegradable polymer that has been used as a local delivery system for proteins and other macromolecules, as reported by Langer, R., and Folkman, J., *Nature (London)*, **263**:797-799 (1976). Others include polyurethanes, polyacrylonitriles, and some polyphosphazenes.

Other delivery systems including films, coatings, pellets, slabs, and devices can be fabricated using solvent or melt casting, and extrusion, as well as standard methods for making composites.

Methods for using the compositions

The compounds can be administered parenterally, i.e. subcutaneously, intramuscularly, intracerebroventricularly, or intravenously and, alternatively, intrathecally. Preferably, the compositions are applied locally, by injection or via controlled delivery devices. Such devices include liposomes, microparticles

(including microspheres and microcapsules), and other release devices and forms that provide controlled, prolonged, sustained or pulsed, delivery.

The appropriate dosages will depend upon the route of administration and the treatment indicated, and can be readily determined by one skilled in the art. Preferably, the concentration of each agent is at a minimum of 150% of its concentration in serum via appropriate oral, i.v. or i.m. route. In the event that systemic administration of the cytotoxic agent at an effective dose is neuro-toxic, organ toxic or fatal, its local application with an immunostimulant offers efficacy with lessen or no adverse effects. Conversely, in the event that systemic administration of the immunostimulant at an effective dose is harmful or fatal, its local application with a cytotoxic compound also offers efficacy with little or no adverse effects. Dosages are generally initiated at lower levels and increased until desired effects are achieved.

The present invention is further described by the following non-limiting examples.

Example 1: Hydroxyurea and *M. Bovis*

Mix four grams of hydroxyurea (USP Grade) well with one gram of lyophilized *Mycobacterium bovis*. Rods of 3 mm (length) x 1 mm (diameter) can be formed by molding. A rod can be inserted into a melanoma via a pre-formed needle hole.

Example 2: 5-FU and Freund's Complete Adjuvant

Mix one gram of 5-fluorourea (USP Grade) with two grams of Freund complete adjuvant to make a paste. The paste can be applied into a slit into melanom. Then a bandage would be placed on the treated site.

Example 3: *M. phlei* and Bleomycin in Glycerin Carrier

Mix two (2) grams of lyophilized *Mycobacterium phlei*, five tenth of a gram of bleomycin (USP Grade) and five (5) grams of glycerin (USP Grade). One (1) gram of the mixture can be delivered to the inside and near a breast cancer mass using a spinal needle during ultrasound imaging.

Example 4: Lipid A and Paclitaxel in Polysorbate 80 Carrier

Mix ten micrograms of lipid A, five grams of paclitaxel and four grams of polysorbate 80. One gram of the mixture can be delivered to the inside and near an ovarian cancer mass using a laparoscopic method.

Example 5: Pyrogen and Cisplatin in Hydroxyapatite Powder

Mix five hundred micrograms of pyrogen, five grams of Cisplatin, and five grams of hydroxyapatite powder. The mixture can be molded under pressure to form a rod of 5 mm (length) x 3 mm (diameter). A rod can be placed into a pre-drilled, 3.5 mm hole on a cancerous bone.

Example 6: Muramyl Dipeptide BiCNU in Gelatin.

Mix five hundred micrograms of muramyl dipeptide, two hundred milligrams of Camustine (BiCNU), three hundred milligrams of gelatin and ten milliliters of water at eighty five degrees centigrade to a consistent mixture. Cool the mixture to 37° C, package and store it. Peri-operatively, ten milliliters of the mixture can be placed in the cavity inside the brain where a glioblastoma was surgically removed.

Example 7: Mitomycin and BCG in Triton-X100 Solution.

Mix one gram of mitomycin and 4 grams of lyophilized *Bacille calmette-guerin* (BCG) with five milliliters of Triton-X100. One-tenth of a millimeter of the mixture can be delivered to the inside of a poly of the colon through a colonoscopy.

Example 8: GM-CSF and Sulfadiazine in a Cream.

Drop-wise, one ml of sterile solution containing 2.5 micrograms of granulocyte-macrophage colony stimulating factor is mixed into 20 grams of commercially available Silvadine® cream that contains 0.2 gram of anti-bacterial silver sulfadiazine. When the final cream becomes consistent, it can be applied directly on a chronic wound sepsis.

Example 9: GM-CSF and Antibiotic in an Ointment.

One microgram of granulocyte-macrophage colony stimulating factor, 500 units of USP-grade bacitracin zinc, 4.0 milligrams of USP-grade neomycin sulfate and 10,000 units of USP-grade Polymyxin B sulfate are mixed with one

gram of USP-grade ointment that contains butylparaben, cholesterol, methyparaben, microcrystalline wax, mineral oil, and white petroleum. The final ointment mixture can be applied directly on a wound sepsis.

Example 10: Valrubicin and BCG in an Ointment.

One hundred milligrams of valrubicin and 1×10^9 CFU BCG are added to 0.5 ml of ethanol and mixed well. Five hundred milliliters of USP-grade ointment are mixed into the valrubicin-BCG-ethanol mixture. The final ointment can be applied directly onto skin or mucous membrane lesions caused and infected by herpes virus.

Example 11: Valrubicin, Cisplatin and BCG Solution

Fifty milligrams of valrubicin, fifty milligrams of cisplatin and 1×10^9 CFU BCG are added to 0.5 ml of ethanol and mixed well. An aliquot of 50 microliters can be injected into a melanoma lesion.

Example 12: Cytobine, Paclitaxel, Doxorubicin and GM-CSF

Five micrograms of IL-3, 0.5 milligram of paclitaxol, 2 milligram of doxorubicin HCl and 1 microgram of granulocyte-macrophage colony stimulating factor are added to 1.0 ml of water for injection. An aliquot of 100 microliters can be injected into a breast cancer mass.

It is believed that the combination of cytotoxic agent and immunostimulants is superior in many ways to the methods of prior art. The local level of the cytotoxic molecule will be much higher than that found in patient's serum after oral, i.m. or i.v. administration at normal dosage. It is believed it will destroy the local cancer mass within much shorter period of time than normal administration. This rapid cell death will occur while large numbers of the immunostimulant molecules are still at the site. The dead cells' surface components and/or contents will, therefor, be presented by the immunostimulant molecules to the cellular or humoral immune system as antigens. As a result of the adjuvant, immunostimulant effects, the animal will produce large quantities of antibodies, interleukins, T-cells, dendritic cells, etc,

that specifically target all antigenic components of cancer cells in distant locations.

Publications cited herein and the material for which they are cited are specifically incorporated by reference. Modifications and variations of the present invention will be obvious to those skilled in the art from the foregoing detailed description and are intended to be encompassed by the following claims.

What is claimed is:

1. A composition comprising:
a cytotoxic compound; and an immunostimulant which enhances or elicits as immune responses in a host animal, in a pharmaceutically acceptable vehicle for local delivery.
2. The composition of claim 1, wherein the cytotoxic compound is selected from the group consisting of alkylating agents, antibiotics, antifungals, antiamebics, antiseptics, antiviral agents, antimetabolites, inorganic and organic compounds, metal chelators, enzymes, enzyme inhibitors, hormones and hormone analogs, platinum complexes, and other antineoplastic compounds.
3. The composition of claim 1, wherein the immunostimulant is selected from the group consisting of mucopolysaccharides, pyrogens, exotoxins, bacterial cell walls and fragments thereof, DNA/RNA, microorganisms, plant allergens, mammalian cytokines, animal allergens, and synthetic immunostimulants.
4. The composition of claim 2, wherein the alkylating agent is selected from the group consisting of nitrosoureas, nitrogen mustards, methanesulfonates, epoxides, ethylenimine, and plant-derived alkaloids.
5. The composition of claim 2, wherein the antibiotic is selected from the group consisting of aminoglycosides, amphenicols, ansamycins, beta-lactams, lincosamides, macrolides, tetracyclines, aclacinomycins, actinomycin F1, anthramycin, bleomycins, chromomycins, dactinomycin, daunorubicin, doxorubicin, idarubicin, mitomycins, porfiromycin, streptoigrin, tubercidin, and zinostatin.
6. The composition of claim 2, wherein the antimetabolite is selected from the group of folic acid analogs, purine analogs, pyrimidine analogs, Triphosphate (2-fluoro-ara-ATP), 5-Fluorouracil, pentosatin, and hydroxyurea and their derivatives.
7. The composition of claim 2, wherein the inorganic compound is

selected from the group consisting of hydrochloric acid, sodium hydroxide, silver nitrate, sulfuric acid, and cesium chloride.

8. The composition of claim 2, wherein the organic compound is selected from the group consists of acetic acid, formic acid, L-ascorbic acid, ethanol, isopropanol, and DMSO.

9. The composition of claim 2, wherein the metal chelator is selected from the group consisting of EDTA, deferoxamine, sodium ditiocarb, calcium disodium edetate, disodium edetate, sodium edetate, trisodium edetate, penicillamine, calcium trisodium pentetate, pentetic acid, succimer, and trientine and their derivatives.

10. The composition of claim 2, wherein the enzyme is asparaginase.

11. The composition of claim 2, wherein the antiviral agent is selected from the group consisting of purines/pyrimidinones, acemannan, acetylleucine monoethanolamine, amantadine, amidinomycin, delavirdine, foscarnet sodium, indinavir, interferons, kethoxal, Iysozyme, methisazone, moroxydine, nevirapine, podophyllotoxin, ribavirin, rimantadine, ritonavir, saquinavir, stallimycin, statolon, tromantadine, and xenazoic acid.

12. The composition of claim 2, wherein the antineoplastic compound is selected from the group consisting of aceglatone, adozelesin, altretamine, aminopterin, amsacrine, anthracycline, AR102, Arimidex, 5-azacytidine, 5-aza-2-deoxycytidine, betamethasone sodium, bisnafide, bisantrene, 2B1 bispecific murine Mab, bizelestin, Bropirimine, bryostatin 1, BUDR, Campath-1H, capecitabine, chloroquinoxaline sulfonamide, CI-980, cordycepin, crisnatol, cytotoxic cytokines, defosfamide, demecolcine, diaziquone, diethyl homospermine, dihydro-5-azacytidine (DHAC), distamycin (tallimustine), [alpha]-difluoromethyl-ornithine (DFMO), docetaxel, 5-or6-{[2,3-bis(hexadecanoyloxy)propyl]phosphonato} ethylcarbamoyl-3,6-O,O-bis(2-nitrobenzyl)-fluorescein sodium salt (DPPE), droloxifene, DTIC-DME, EGF fusion toxin, anti-EGFr chimeric Mab, EGM fusion toxin, eflornithine, elliptinium acetate, estramustine phosphate sodium, amifostine, etoposide,

etoglucid, exemestane, fenretinide, filgrastim, Filmix, gallium nitrate, gemcitabine, glucosamylmuramyl tripeptide dipalmitoylglycerol, guanine arabinoside, homoharringtonine, hydroxyurea, idarubicin, idoxifene, A, irinotecan, idoxuridine(IUDR), LDI-200 letrozole, liarozole, linomide roquinimex, lonidamine, miltefosine, mitotane, Matulane, MDX-447, MDX-H210, mitalactol, mitoguazone, mitoxantrone, mopidamol, Navelbine, nitracine, octreotide pamoate, oxaliplatin, paclitaxel, peldesine, pentostatin, phenamet, phenylacetate, N-phosphonoethyl-L-aspartic acid, piritrexim, plicamycin, podophyllinic acid 2-ethyl-hydrazide, procarbazine, prazine, Radinyl etanidazole, radiolabeled Mab CC-49, razoxane, 9-cis-retinoic acid, RMP-7, SU101, sobuzoxane, spirogermanium, TBC-CEA, temozolomide, teniposide, tenuazonic acid, thalidomide, tirapazamine, topotecan, toremifene, triaziquone, 2,2,2-trichlorotriethylamine, VX-710, timetrexate glucuronate, tretinoin, and urethan.

13. The composition of claim 2, wherein the hormone or hormone analog is selected from the group consisting of anastrozole, flutamide, nilutamide, tamoxifen sulfate, diethylstilbestrol, and chlorotrianisene.

14. The composition of claim 3, wherein the mucopolysaccharide is selected from the group consisting of chondroitin sulfates A, B, and C, hyaluronic acid, and other glucosaminoglycans.

15. The composition of claim 3, wherein the pyrogen is selected from the group consisting of chemical pyrogens, microbial endotoxins, fragments of microbial endotoxins and lipid A.

16. The composition of claim 3, wherein the bacterial cell wall fragments are pieces and components of the cell wall of microorganisms.

17. The composition of claim 3, wherein the DNA and RNA are bacterium DNA fragments containing CG motif, RNAs, or their complexes.

18. The composition of claim 16, wherein the microorganism is selected from the group consisting of selected from the group consisting of *Bordatella pertussis*, *Corynebacterium parvum*, Eubacterials, *Mycobacterium*

avium, *Mycobacterium bovis*, *Mycobacterium fortuitum*, *Mycobacterium kansasii*, *Mycobacterium phlei*, *Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, *Mycobacterium vaccae*, *Nocardia rubra*, *Nocardia asteroides*, and *Rhodococcus*.

19. The composition of claim 3, wherein the plant allergen is derived from a plant selected from the group consisting of *Acer negundo*, *Agrostis alba*, *Alnus incana*, *Ambrosia elatior*, *Artemisia tridentata*, *Betula alba*, *Bromus inermis*, *Cynodon dactylon*, *Dactylis glomerata*, *Fraxinus pennsylvanica*, *Iva xantifolia*, *Juglans nigra*, *Juniperus scopulorum*, *Kochia scoparia*, *Poa pratensis*, *Populus nigra italica*, *Quercus rubra*, *Secale cereale*, *Sorghum halepense*, *Ulmus pumilazea mays*, poison hemlock, poison ivy, poison oak,

20. The composition of claim 3, wherein the animal allergen is derived from an animal selected from the group consisting of ants, bees, centipedes, cone shell, Gila monster, jellyfishes, keyhole limpet, kissing bug, millipedes, mosquitoes, puss caterpillar, scorpions, scorpion fish, sea anemone, sea urchins, snakes, spiders, spotted octopus, starfishes, stonefish, and weever fish.

21. The composition of claim 3, wherein the synthetic immunostimulants are selected from the group consisting of Levamisole, Isoprinosine, and their derivatives.

22. The composition of claim 3, wherein the exotoxins are microbial exotoxins or fragments thereof.

23. The composition of claim 16, wherein the pieces of the cell wall of microorganisms are generated by mechanical shearing, thermal denaturation or chemical disruption of microbial cell walls.

24. The composition of claim 16, wherein the components of the cell wall of microorganisms are selected from the group consisting of lipopolysaccharide, lipoteichoic acid, peptidoglycan, muramyl dipeptide (N-acetylmuramyl-L-alanyl-D-isoglutamine), derivatives of mycolic acids, and their complexes.

25. The composition of claim 3, wherein the exotoxins are selected from the group consisting of mycotoxins, diphtheria exotoxin, tetanus toxoid, botulinus toxoid.
26. The composition of claim 1 wherein the composition further comprises a controlled or sustained release carrier.
27. The composition of claim 1 wherein the composition comprises a carrier for topical application.
28. The composition of claim 3, wherein the macrophage stimulating factor is selected from the group consisting of granulocyte-macrophage colony stimulating factors and macrophage inflammatory proteins.
29. The composition of claim 3, wherein the mammalian cytokine is selected from the group consisting of interferons and interleukins.
30. A method for treating tissue affected by microbial or viral infection or cancerous tissue comprising applying a cytotoxic agent in combination with an immunostimulating agent in a pharmaceutically acceptable carrier to site in a patient in need of treatment thereof.
31. The method of claim 30 wherein the cytotoxic agent and the immunostimulant are applied simultaneously.
32. The method of claim 30 wherein the cytotoxic agent and the immunostimulant are applied sequentially.
33. The method of claim 30 wherein the cytotoxic compound and the immunostimulant are applied locally to the diseased tissue.
34. The method of claim 30 wherein the cytotoxic compound and the immunostimulant are applied in a controlled or sustained delivery carrier.